Physical Activity Status and Adverse Age-Related Differences in Coagulation and Fibrinolytic Factors in Women

Christopher A. DeSouza, Pamela Parker Jones, Douglas R. Seals

Abstract—Adverse changes in coagulation and fibrinolytic factors are thought to contribute to the increased risk of cardiovascular disease and atherothrombosis with age. We tested the hypothesis that such age-related changes in specific coagulation and fibrinolytic factors are absent in physically active women. Resting levels of plasma fibrinogen, tissue-type plasminogen activator (t-PA) antigen and activity, plasminogen activator inhibitor-1 (PAI-1) antigen and activity, and fibrin D-dimer were measured in 24 healthy premenopausal women: 11 sedentary (aged 28±1 years; Pre-S) and 13 physically active (aged 30±1 years; Pre-PA) and in 27 healthy postmenopausal women: 14 sedentary (aged 61±1 years; Post-S) and 13 physically active (aged 58±1 years; Post-PA). Post-S had higher (P<.05) fibrinogen, t-PA antigen, PAI-1 antigen, PAI-1 activity, and fibrin D-dimer levels and lower t-PA activity than Pre-S. Post-PA demonstrated lower (P<.01) plasma fibrinogen, t-PA antigen, PAI-1 antigen, and PAI-1 activity and higher (P<.01) t-PA activity levels than Post-S. In addition, plasma fibrin D-dimer levels tended (P=.06) to be lower in Post-PA than in Post-S. Although plasma levels of fibrinogen and fibrin D-dimer in Post-PA were lower than in Post-S, they were higher (P<.05) than in Pre-PA. Importantly, however, the fibrinolytic profile of Post-PA did not differ from that of Pre-PA. The results of the present study demonstrate that the adverse age-associated differences in plasma fibrinogen concentrations and the endogenous fibrinolytic system in sedentary healthy women are either attenuated or absent in highly physically active women. The smaller or absent age-related differences in coagulation and fibrinolytic factors in women who habitually exercise may represent an important mechanism contributing to their lower age-related increase in both cardiovascular disease and atherothrombotic events. Future studies need to determine whether women who are moderately active would demonstrate the same favorable hemostatic profile. (Arterioscler Thromb Vasc Biol. 1998;18:362-368.)

Key Words: aging ■ exercise ■ fibrinogen ■ fibrinolytic system ■ fibrin D-dimer

Advancing age is associated with an increased risk of CVD in general and atherosclerotic vascular disease in particular.1 In women, the incidence of both CVD and thrombosis increases after the onset of menopause.2–3 It has been suggested that age-related changes in coagulation and fibrinolytic factors contribute to the increased risk of atherothrombotic events in postmenopausal women by accelerating the atherosclerotic process and promoting thrombus formation.4–6 Indeed, higher plasma concentrations of fibrinogen4 and fibrin D-dimer,7 both markers of thrombogenic risk, and reduced endogenous fibrinolytic activity8 have been reported in healthy postmenopausal compared with premenopausal women.

In contrast to aging, regular physical activity is associated with favorable coagulation and fibrinolytic function.8–10 We have previously shown that physically active postmenopausal women demonstrate lower plasma fibrinogen8 concentrations and improved fibrinolytic function evidenced by lower t-PA antigen and PAI-1 activity8 levels compared with age-matched sedentary control subjects. However, whether regular physical activity prevents adverse age-related changes in coagulation and fibrinolytic factors is unknown. If so, it could contribute to the smaller age-related increases in both CVD and atherothrombotic events observed in physically active compared with sedentary women.11

Accordingly, the primary purpose of the present investigation was to test the hypothesis that adverse age-related differences in specific coagulation and fibrinolytic factors are absent in physically active women. To systematically test this hypothesis, we used a cross-sectional model to first document the detrimental age-associated differences in plasma fibrinogen, t-PA antigen, t-PA activity, PAI-1 antigen, PAI-1 activity, and fibrin D-dimer levels in healthy sedentary women. We then measured the aforementioned hemostatic factors in corresponding groups of endurance-trained women.

Methods

Subjects
Fifty-one healthy women were studied: 11 Pre-S, 13 Pre-PA, 14 Post-S, and 13 Post-PA women. The Pre-PA and Post-PA women...
were matched for age-adjusted running performance as described previously by our laboratory\(^2\) and ran 58±7 and 48±3 km/wk, respectively. The physically active women were recruited from various running clubs throughout the Boulder area and from participants in the Boulder Boulder, the second largest 10-km road race in the United States. The sedentary subjects were recruited through local newspaper advertisements and had not participated in a regular aerobic exercise program for at least 1 year before the start of the study. All of the postmenopausal women were at least 2 years postmenopausal (range, 2 to 23 years), and an equal number of each group were taking estrogen-based hormone supplements (8 sedentary and 8 physically active). All premenopausal women were eumenorrheic, as assessed by self-report of menstrual cycles, not taking oral contraceptives, and studied during the follicular phase of their menstrual cycle. All subjects were free of overt disease, as assessed by medical history. The postmenopausal women were further evaluated for clinical evidence of cardiovascular disease with a physical examination and resting and maximal exercise electrocardiograms. No subjects were on medication other than hormone replacement, and all subjects were nonsmokers.

Measurement of Coagulation and Fibrinolytic Variables

Plasma fibrinogen levels were measured using the method of Clauss. Plasma t-PA antigen, PAI-1 antigen, and fibrin d-dimer were determined by using an enzyme-linked immunosorbent assay (American Bioproducts). t-PA activity and PAI-1 activity were measured using an amidolytic method (Chromogenix). t-PA activity is expressed in international units and PAI-1 activity in arbitrary units. One AU is defined as the amount of inhibitor that inhibits 1 IU of t-PA per milliliter of plasma.19 Intra-assay and interassay coefficients of variation were 8.6% and 7.5%, respectively, for t-PA antigen; 6.9% and 7.4% for t-PA activity; 8.9% and 8.2% for PAI-1 antigen; 3.8% and 3.5% for PAI-1 activity; and 4.7% and 6.4% for fibrin d-dimer.

Statistical Analysis

The influence of both age and physical activity on all variables was determined by a multifactor ANOVA (age×physical activity). When indicated by a significant F value, specific mean comparisons were performed to identify significant group differences. Simple and forward stepwise multiple regression analyses and partial correlation coefficients were calculated to determine relations between the specific coagulation and fibrinolytic variables and anthropometric, hemodynamic, and metabolic variables. ANCOVA was used to statistically adjust for the influence of each independent determinant identified by the stepwise multiple regression analysis. All data are expressed as mean±SE. Statistical significance was set at \(P<.05\).

Results

Physical Characteristics

Table 1 shows the physical characteristics of the subjects. Body mass, percent body fat, and BMI were higher \((P<.05)\) in Pre-PA and Post-S than in their age-matched physically active counterparts. In addition, percent body fat and BMI were higher \((P<.01)\) in Post-S than in Pre-S and Post-PA than in Pre-PA. There were no differences in fat-free mass among the four groups. WHR was higher \((P<.01)\) in Post-S than in any other group. The Pre-PA demonstrated the highest \((P<.01)\) and Post-S the lowest \((P<.01)\) VO\(_2\)max of all groups. Systolic blood pressure was higher \((P<.01)\) in the postmenopausal women relative to their respective premenopausal control subjects. There were no differences in diastolic blood pressure among the four groups.
Table 1. Physical Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sedentary</th>
<th></th>
<th>Physically Active</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premenopausal (n=14)</td>
<td>Postmenopausal (n=11)</td>
<td>Premenopausal (n=13)</td>
<td>Postmenopausal (n=13)</td>
</tr>
<tr>
<td>Age, y</td>
<td>28.4±1.4</td>
<td>30.1±1.1</td>
<td>54.3±1.8†</td>
<td>58.1±1.3*</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>62.0±3.8</td>
<td>68.9±2.8</td>
<td>58.8±1.4†</td>
<td>58.8±1.4†</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>27.4±2.3</td>
<td>39.9±2.1†</td>
<td>16.3±1.4†</td>
<td>24.5±1.4††</td>
</tr>
<tr>
<td>Fat free mass, kg</td>
<td>44.3±1.7</td>
<td>40.9±1.0</td>
<td>45.3±1.3</td>
<td>44.3±1.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.5±1.3</td>
<td>26.5±0.9*</td>
<td>19.6±0.4†</td>
<td>20.9±0.3††</td>
</tr>
<tr>
<td>WHR</td>
<td>0.75±0.01</td>
<td>0.83±0.02*</td>
<td>0.74±0.01</td>
<td>0.74±0.01†</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>99±2</td>
<td>114±3*</td>
<td>101±3</td>
<td>110±3*</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>73±2</td>
<td>75±2</td>
<td>70±2</td>
<td>74±2</td>
</tr>
<tr>
<td>VO₂ max, mL/min/kg</td>
<td>34.3±1.6</td>
<td>22.2±1.2*</td>
<td>53.7±1.3†</td>
<td>38.2±1.6††</td>
</tr>
</tbody>
</table>

VO₂ max indicates maximal oxygen consumption; SBP, systolic blood pressure; and DBP, diastolic blood pressure. Values are mean±SE.
*P<.01 vs premenopausal women of same physical activity status. †P<.01 vs age-matched sedentary.

Coagulation and Fibrinolytic Variables

Fibrinogen

There was a significant main effect of both age and physical activity on plasma fibrinogen levels. The age-related difference was apparent in both the sedentary and physically active women, as plasma fibrinogen levels were higher in Post-S than in Pre-S (2.85±0.09 versus 2.28±0.08 g/L; P<.01) and Post-PA than in Pre-PA (2.49±0.09 versus 2.18±0.07 g/L; P=0.2; Fig 1). The physical activity–related difference, however, was observed only in the older subjects, with lower (P=.002) plasma fibrinogen levels in Post-PA than in Post-S.

Fibrin d-Dimer

Similar to fibrinogen, plasma fibrin d-dimer levels were higher in Post-S than in Pre-S (335.0±53.6 versus 113.0±11.1 ng/mL) and Post-PA than in Pre-PA (265.2±24.6 versus 123.7±11.4 ng/mL; Fig 1). However, although the Post-PA tended (P=.06) to have lower plasma fibrin d-dimer levels compared with the Post-S, there was no significant effect of physical activity status on plasma fibrin d-dimer levels.

Correlations and ANCOVA

Significant univariate correlations were observed between the specific coagulation and fibrinolytic variables and body composition, blood pressure, and metabolic characteristics of the subjects (Table 2). Multiple stepwise regression analysis revealed that percent body fat (R²=.31) and plasma total cholesterol (R²=.31) were the primary correlates of plasma fibrinogen and fibrin d-dimer concentrations in the overall study population, respectively. For the fibrinolytic variables, percent body fat (R²=.51) and BMI (R²=.23) were the strongest correlates of t-PA antigen and t-PA activity, respectively whereas BMI (R²=.53) and waist circumference (R²=.46) were the strongest correlates of PAI-1 antigen and PAI-1 activity, respectively. After statistically controlling for each primary determinant, the differences among the groups were no longer significant.

Discussion

There are three important findings of the present study. First, although physical activity status does not appear to prevent an age-related increase in plasma fibrinogen concentrations, the magnitude of the elevation with age is only ≈50% as great in
habitually exercising compared with sedentary women. Second, adverse age-related differences in the fibrinolytic system were not observed in the physically active women, suggesting that regular physical activity may prevent the decline in fibrinolytic function observed with age in sedentary women. Finally, similar age-related increases in plasma fibrin d-dimer levels were observed in both the physically active and sedentary women.

Fibrinogen
Plasma fibrinogen, the circulating precursor of fibrin, is a major independent risk factor for atherosclerotic CVD in postmenopausal women. Plasma fibrinogen levels have been shown to increase with age and to be related to the incidence of future coronary events in both healthy and diseased populations. In addition, we and others have previously reported that plasma fibrinogen concentrations are lower in physically active than in sedentary postmenopausal women. The present findings confirm and extend these earlier observations by demonstrating that the age-related elevation in plasma fibrinogen levels is twice as great in the sedentary (+0.57 g/L) as in the physically active (+0.31 g/L) women. Importantly, the lower plasma fibrinogen levels in the physically active women would appear to be associated with a reduction in CVD risk.

TABLE 2. Correlations (r) Between the Coagulation and Fibrinolytic Variables and Anthropometric, Hemodynamic, and Metabolic Data in the Overall Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fibrinogen</th>
<th>Fibrin d-Dimer</th>
<th>t-PA Antigen</th>
<th>t-PA Activity</th>
<th>PAI-1 Antigen</th>
<th>PAI-1 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass</td>
<td>.45†</td>
<td>.33*</td>
<td>.47†</td>
<td>−.38†</td>
<td>.57†</td>
<td>.49†</td>
</tr>
<tr>
<td>BMI</td>
<td>.42†</td>
<td>.41†</td>
<td>.66†</td>
<td>−.48†</td>
<td>.73†</td>
<td>.62†</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>.52†</td>
<td>.53†</td>
<td>.64†</td>
<td>−.47†</td>
<td>.69†</td>
<td>.66†</td>
</tr>
<tr>
<td>WHR</td>
<td>.34*</td>
<td>.40†</td>
<td>.53†</td>
<td>−.33*</td>
<td>.50†</td>
<td>.52†</td>
</tr>
<tr>
<td>Percent fat</td>
<td>.59†</td>
<td>.48†</td>
<td>.69†</td>
<td>−.40†</td>
<td>.66†</td>
<td>.60†</td>
</tr>
<tr>
<td>VO2max</td>
<td>−.54†</td>
<td>−.46†</td>
<td>−.67†</td>
<td>−.39†</td>
<td>−.57†</td>
<td>−.62†</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>.44†</td>
<td>.55†</td>
<td>.55†</td>
<td>−.16</td>
<td>.25</td>
<td>.34*</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>.36*</td>
<td>.32*</td>
<td>.14</td>
<td>−.19</td>
<td>.27</td>
<td>.29*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>.50†</td>
<td>.57†</td>
<td>.55†</td>
<td>−.28</td>
<td>.35*</td>
<td>.30*</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>.09</td>
<td>.11</td>
<td>.03</td>
<td>−.08</td>
<td>.10</td>
<td>.04</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>.53†</td>
<td>.53†</td>
<td>.55†</td>
<td>−.28*</td>
<td>.31†</td>
<td>.31*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>.15</td>
<td>.39†</td>
<td>.46†</td>
<td>−.28*</td>
<td>.25</td>
<td>.13</td>
</tr>
<tr>
<td>Glucose</td>
<td>.34*</td>
<td>.33*</td>
<td>.43†</td>
<td>−.36†</td>
<td>.37†</td>
<td>.39†</td>
</tr>
<tr>
<td>Insulin</td>
<td>.09</td>
<td>.02</td>
<td>.45†</td>
<td>−.32*</td>
<td>.48†</td>
<td>.42†</td>
</tr>
<tr>
<td>SI</td>
<td>.52†</td>
<td>.39†</td>
<td>.43†</td>
<td>.18</td>
<td>.25</td>
<td>.38†</td>
</tr>
</tbody>
</table>

*P<.05; †P<.01.

SI indicates estimate of insulin sensitivity from a frequently sampled intravenous glucose tolerance test.
fibrinogen levels increased the risk of an initial cardiovascular event by 25%. Thus, although there was a significant increase in plasma fibrinogen levels with age in both the sedentary and physically active groups in the present study, the magnitude of the increase and the associated cardiovascular risk appears to be lower in physical activity women. Lower plasma fibrinogen concentrations may reduce the risk of atherosclerosis and thrombosis by favorably altering blood viscosity and platelet adhesion and aggregation and by limiting intravascular fibrin formation and deposition.

The mechanisms by which habitual physical activity may attenuate the age-related increase in plasma fibrinogen concentrations are not clear. It has been suggested that the favorable association between plasma fibrinogen levels and regular exercise are likely due, at least in part, to lower body fatness. Indeed, in the present study, percent body fat was the primary determinant of plasma fibrinogen level, accounting for approximately one third of the variability.

Fibrinolytic System

The hemostatic mechanism responsible for the proteolytic degradation of intravascular fibrin deposition is the fibrinolytic system. This enzymatic pathway maintains vascular patency by converting the inactive proenzyme plasminogen to the active enzyme plasmin, which lyses fibrin into soluble degradation products. Both clinical and epidemiological data have suggested that reduced endogenous fibrinolytic activity, characterized by increased t-PA antigen, PAI-1 antigen, and PAI-1 activity and reduced t-PA activity, is a major contributor to both the development and severity of atherothrombosis.

Our finding of impaired fibrinolytic function with age in sedentary women is consistent with previous reports and supports the hypothesis that hypofibrinolysis may contribute to the increased risk of atherothrombotic events with age in women. In addition, the lower t-PA antigen and PAI-1 activity observed in the physically active postmenopausal women relative to the sedentary control subjects confirm our previous observations in middle-aged and older women. Importantly, the results of the present study significantly extend our previous findings by showing, for the first time, that the adverse age-related differences in the fibrinolytic system observed in sedentary women are absent in physically active women. In contrast to their sedentary peers, there were no age-related increases in t-PA antigen, PAI-1 antigen, and PAI-1 activity or a concomitant decrease in t-PA activity in the physically active women. In fact, the hyperfibrinolytic state observed in the physically active postmenopausal women was similar to that observed in their active premenopausal peers. As a result, the molar concentration ratio of active t-PA to active PAI-1, an index of fibrinolytic potential defined as the ability to respond to a stimulus and lyse fibrin, was almost identical in the premenopausal and postmenopausal endurance-trained women.

Thus, these data suggest that adverse age-related differences in the fibrinolytic system may not be a primary effect of aging. Rather, such changes may be due, at least in part, to age-related reductions in physical activity and associated increases in body weight and fatness. In the present study, percent body fat, BMI, and waist circumference were the primary determinants of the fibrinolytic variables. These observations are consistent with previous studies that have reported an association between the fibrinolytic system and total and abdominal body fatness and support the suggested link between adiposity and thrombogenic risk.

In the present study, the lower t-PA antigen, PAI-1 antigen, and PAI-1 activity and the higher t-PA activity observed in the physically active compared with sedentary postmenopausal women may be of significant clinical importance. Recent findings suggest that t-PA antigen is both a biological marker of subclinical atherosclerosis and a strong predictor of future acute myocardial infarction in healthy individuals. In addition, because t-PA is synthesized primarily by endothelial cells, it has been proposed that elevated levels may also reflect endothelial cell inflammation and damage. Furthermore, PAI-1, the major determinant of fibrinolytic activity, has been suggested to play an important role in the development of atherothrombosis. Elevated PAI-1 gene expression, localization, and production have been reported to occur in injured atherosclerotic arteries. In contrast, elevated t-PA activity is associated with a decreased risk of cardiovascular events. Thus, the absence of age-associated impairments in the fibrinolytic system in the physically active women may be mechanistically involved in the lower incidence of atherothrombotic events observed in this population.

Fibrin D-Dimer

Fibrin D-dimer is a specific degradation product of the enzymatic action of plasmin on cross-linked fibrin. Elevated plasma levels have been suggested to be a sensitive marker of intravascular fibrin formation and the extent and severity of underlying atherosclerosis. In the present study, there was a significant influence of age on plasma fibrin D-dimer concentrations in both the sedentary and physically active women. Our finding of higher plasma fibrin D-dimer levels in the sedentary postmenopausal compared with premenopausal women is consistent with previous studies and supports the premise of increased fibrin formation and deposition with age. An original finding of the present study, however, was that regular physical activity does not appear to influence the effect of age on plasma fibrin D-dimer levels. Although the physically active postmenopausal women demonstrated lower absolute plasma fibrin D-dimer levels than their sedentary age-matched peers, they had significantly higher fibrin D-dimer levels than their younger counterparts. However, given the fact that the age-related increase in plasma fibrin D-dimer levels in the postmenopausal groups occurred under two very different fibrinolytic conditions, ie, low fibrinolytic activity in the sedentary group and high fibrinolytic activity in the physically active group, it is possible that the elevated levels of fibrin D-dimer may be reflecting two different physiological conditions. In the sedentary postmenopausal women, the age-related increase in plasma fibrin D-dimer levels may reflect a prothrombotic state characterized by numerous small amounts of fibrin degradation products resulting from chronic extensive intravascular fibrin formation and degradation. On the other hand, the elevated levels of plasma fibrin D-dimer found in our physically active postmenopausal group may
reflect increased fibrin turnover resulting from elevated levels of circulating fibrin.44

Limitations

The primary limitation of this study is its cross-sectional design and the inherent possibility that constitutional factors may have influenced our findings. However, the plasma concentrations of the hemostatic factors presented herein are consistent with previous studies involving both sedentary4,47,48 and physically active10 populations. In addition, the favorable plasma levels of fibrinogen, t-PA antigen, t-PA activity, and PAI-1 activity associated with regular physical activity in the present study are similar to those reported in response to endurance exercise training in older men.49 Nevertheless, to determine the effects of physical activity per se on coagulation and fibrinolytic factors in postmenopausal women, longitudinal studies in this population will need to be performed. Second, it is important to note that the physically active subjects in the present study were highly endurance-trained athletes; it was our purpose to use these women to address the physiological question posed. As such, we are unable to comment on whether women who are moderately active would demonstrate the same favorable hemostatic profile. Given the clinical importance of coagulation and fibrinolysis to thrombogenic risk, future studies are needed to address this issue.

Conclusion

In conclusion, the results of the present study indicate that the adverse age-associated differences in plasma fibrinogen concentrations and the endogenous fibrinolytic system in seden-

ary healthy women are either attenuated or absent in highly physically active women. These differences may play an important role in the smaller age-related increase in the incidence of CVD and thrombosis in physically active com-

pared with sedentary women.

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